

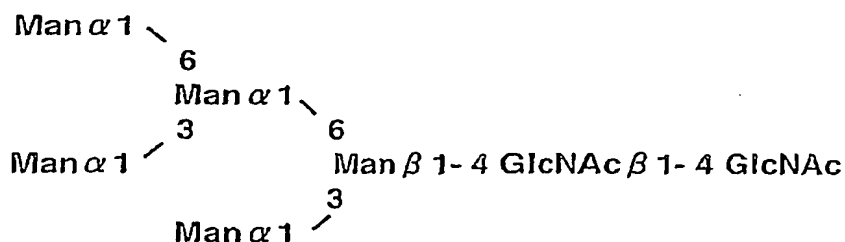
What is claimed is:

1. A process for producing a methylotrophic yeast capable of producing a mammalian type sugar chain, which comprises the steps of:

- 1) disrupting an *OCH1* gene which encodes α -1,6-mannosyl transferase, in a methylotrophic yeast; and
- 2) introducing an α -1,2-mannosidase gene into the yeast and expressing it therein.

2. A process according to claim 1, wherein the mammalian type sugar chain is represented by the following structural formula ($\text{Man}_5\text{GlcNAc}_2$):

Structural Formula 2



3. A process according to claim 1 or 2, wherein the methylotrophic yeast belongs to the genus *Pichia*, *Hansenula*, *Candida*, or *Ogataea*.
4. A process according to claim 1 or 2, wherein the methylotrophic yeast is *Ogataea minuta*.
5. A process according to any one of claims 1 to 4, wherein the methylotrophic yeast is a strain from *Ogataea minuta* strain IFO 10746.
6. A process according to any one of claims 1 to 5, wherein the α -1,2-mannosidase gene is expressed under the control of a methanol-inducible promoter.

7. A process according to claim 6, wherein the methanol-inducible promoter is a promoter of an alcohol oxidase (*AOX*) gene.
8. A process according to claim 7, wherein the alcohol oxidase (*AOX*) gene is from *Ogataea minuta*.
9. A process according to any one of claims 1 to 8, characterized in that the α -1,2-mannosidase gene to be introduced is attached to a yeast endoplasmic reticulum (ER) retention signal (HEDL).
10. A process according to any one of claims 1 to 9, wherein the α -1,2-mannosidase gene is from *Aspergillus saitoi*.
11. A process according to any one of claims 1 to 10, which further comprises a step of transforming a heterologous gene into the yeast.
12. A process according to claim 11, wherein the heterologous gene is transferred using an expression vector and is expressed in the yeast.
13. A process according to claim 12, wherein the expression vector comprises a methanol-inducible promoter.
14. A process according to claim 13, wherein the methanol-inducible promoter is a promoter of an alcohol oxidase (*AOX*) gene.
15. A process according to claim 14, wherein the alcohol oxidase (*AOX*) gene is from *Ogataea minuta*.

16. A process according to claim 12, wherein the expression vector comprises a promoter of a glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) gene.
17. A process according to any one of claims 11 to 16, wherein 20 % or more of N-linked sugar chains produced of the protein encoded by the heterologous gene is the mammalian type sugar chain represented by Structural Formula 2.
18. A process according to any one of claims 11 to 16, wherein 40 % or more of N-linked sugar chains produced of the protein encoded by the heterologous gene is the mammalian type sugar chain represented by Structural Formula 2.
19. A process according to any one of claims 11 to 16, wherein 60 % or more of N-linked sugar chains produced of the protein encoded by the heterologous gene is the mammalian type sugar chain represented by Structural Formula 2.
20. A process according to any one of claims 11 to 16, wherein 80 % or more of N-linked sugar chains produced of the protein encoded by the heterologous gene is the mammalian type sugar chain represented by Structural Formula 2.
21. A process according to any one of claims 11 to 20, wherein the protein encoded by the heterologous gene is from humans.
22. A process according to any one of claims 11 to 21, wherein the protein encoded by the heterologous gene is an antibody or a fragment thereof.
23. A methylotrophic yeast produced by a process according to any one of claims 1 to 22.

24. A process for producing a protein encoded by a heterologous gene, wherein the process comprises culturing the methylotrophic yeast of claim 23 in a medium to obtain the protein encoded by the heterologous gene comprising a mammalian type sugar chain from the culture.
25. A protein comprising a mammalian type sugar chain encoded by the heterologous gene, wherein the protein is produced by the process of claim 24.
26. An orotidine-5'-phosphate decarboxylase (*URA3*) gene DNA encoding an amino acid sequence substantially represented by SEQ ID NO:16.
27. A *URA3* gene DNA comprising a nucleotide sequence substantially represented by SEQ ID NO:15.
28. A recombinant expression vector substantially comprising the gene of claim 26 or 27 or a fragment thereof as a selectable marker.
29. An *Ogataea minuta* strain transformed with the recombinant expression vector of claim 28.
30. An *Ogataea minuta* strain according to claim 29, the strain being from the strain IFO 10746.
31. A phosphoribosyl-amino-imidazole succinocarboxamide synthase (*ADE1*) gene DNA encoding an amino acid sequence substantially represented by SEQ ID NO:28.
32. An *ADE1* gene DNA comprising a nucleotide sequence substantially represented by SEQ ID NO:27.

33. A recombinant expression vector substantially comprising the gene of claim 31 or 32 or a fragment thereof as a selectable marker.
34. An *Ogataea minuta* strain transformed with the recombinant expression vector of claim 33.
35. An *Ogataea minuta* strain according to claim 34, the strain being from the strain IFO 10746.
36. An imidazole-glycerol-phosphate dehydratase (*HIS3*) gene DNA encoding an amino acid sequence substantially represented by SEQ ID NO:100.
37. An *HIS3* gene DNA comprising a nucleotide sequence substantially represented by SEQ ID NO:99.
38. A recombinant expression vector substantially comprising the gene DNA of claim 36 or 37 or a fragment thereof as a selectable marker.
39. A *Ogataea minuta* strain transformed with the recombinant expression vector of claim 38.
40. An *Ogataea minuta* strain according to claim 39, the strain being from the strain IFO 10746.
41. A 3-isopropylmalate dehydrogenase (*LEU2*) gene DNA encoding an amino acid sequence substantially represented by SEQ ID NO:108.
42. A *LEU2* gene DNA comprising a nucleotide sequence substantially represented by SEQ ID NO:107.

43. A recombinant expression vector substantially comprising the gene of claim 41 or 42 or a fragment thereof as a selectable marker.
44. An *Ogataea minuta* strain transformed with the recombinant expression vector of claim 43.
45. An *Ogataea minuta* strain according to claim 44, the strain being from the IFO 10746.
46. An α -1,6-mannosyl transferase (*OCHI*) gene DNA encoding an amino acid sequence substantially represented by SEQ ID NO:43.
47. An *OCHI* gene DNA comprising a nucleotide sequence substantially represented by SEQ ID NO:42.
48. An *Ogataea minuta* strain wherein the gene of claim 46 or 47 has been disrupted.
49. An *Ogataea minuta* strain according to claim 48, the strain being from the strain IFO 10746.
50. A proteinase A (*PEP4*) gene DNA encoding an amino acid sequence substantially represented by SEQ ID NO:52.
51. A *PEP4* gene DNA comprising a nucleotide sequence substantially represented by SEQ ID NO:51.
52. An *Ogataea minuta* strain wherein the gene of claim 50 or 51 has been disrupted.

53. An *Ogataea minuta* strain according to claim 52, the strain being from the strain IFO 10746.
54. A proteinase B (*PRBI*) gene DNA encoding an amino acid sequence substantially represented by SEQ ID NO:58.
55. A *PRBI* gene DNA comprising a nucleotide sequence substantially represented by SEQ ID NO:57.
56. An *Ogataea minuta* strain wherein the gene of claim 54 or 55 has been disrupted.
57. An *Ogataea minuta* strain according to claim 56, the strain being from the strain IFO 10746.
58. A *YPSI* gene DNA encoding an amino acid sequence substantially represented by SEQ ID NO:116.
59. A *YPSI* gene DNA comprising a nucleotide sequence substantially represented by SEQ ID NO:115.
60. An *Ogataea minuta* strain wherein the gene of claim 58 or 59 has been disrupted.
61. An *Ogataea minuta* strain according to claim 60, the strain being from the strain IFO 10746.
62. A process for producing a protein encoded by a heterologous gene, wherein the heterologous gene is transferred into the *Ogataea minuta* strain of claim 60 or 61.

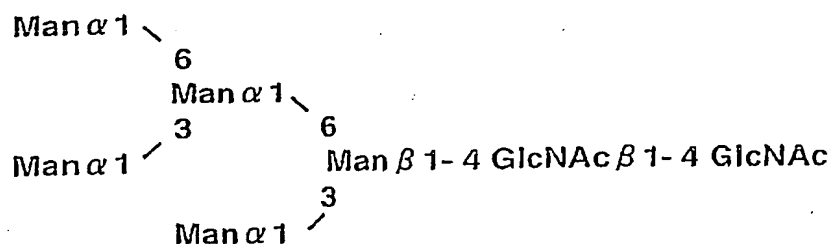
63. A process according to claims 62, wherein the heterologous gene encodes an antibody or a fragment thereof.
64. A process for preventing decomposition of an antibody or a fragment thereof, comprising disrupting a *YPS1* gene in a methylotrophic yeast.
65. A process according to claim 64, wherein the methylotrophic yeast is an *Ogataea minuta* strain.
66. A process according to claim 65, wherein the *Ogataea minuta* strain is from the strain IFO 10746.
67. A process according to any one of claims 64 to 66, wherein class of the antibody is IgG.
68. A process according to claim 67, wherein subclass of the IgG is IgG1.
69. A process according to any one of claims 64 to 68, wherein the antibody is a human antibody.
70. A *KTR1* gene DNA encoding an amino acid sequence substantially represented by SEQ ID NO:64.
71. A *KTR1* gene DNA comprising a nucleotide sequence substantially represented by SEQ ID NO:63.
72. An *Ogataea minuta* strain wherein the gene of claim 70 or 71 has been disrupted.

73. An *Ogataea minuta* strain according to claim 72, the strain being from the strain IFO 10746.
74. An *MNN9* gene DNA encoding an amino acid sequence substantially represented by SEQ ID NO:70.
75. An *MNN9* gene DNA comprising a nucleotide sequence substantially represented by SEQ ID NO:69.
76. An *Ogataea minuta* strain wherein the gene of claim 74 or 75 has been disrupted.
77. An *Ogataea minuta* strain according to claim 76, the strain being from the strain IFO 10746.
78. An alcohol oxidase (*AOX*) gene DNA encoding an amino acid sequence substantially represented by SEQ ID NO:78.
79. An *AOX* gene DNA comprising a nucleotide sequence substantially represented by SEQ ID NO:77.
80. A DNA comprising a promoter of alcohol oxidase (*AOX*)gene which is substantially represented by SEQ ID NO:79.
81. A DNA comprising a terminator of alcohol oxidase (*AOX*) gene which is substantially represented by SEQ ID NO:80.
82. A gene expression cassette comprising the DNA comprising the promoter as defined in claim 80, a heterologous gene, and the DNA comprising the terminator as defined in claim 81.

83. A recombinant expression vector comprising the gene expression cassette of claim 82.
84. An *Ogataea minuta* strain transformed with the recombinant expression vector of claim 83.
85. An *Ogataea minuta* strain according to claim 84, the strain being from the strain IFO 10746.
86. A glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene DNA encoding an amino acid sequence substantially represented by SEQ ID NO:6.
87. A glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene DNA comprising a nucleotide sequence substantially represented by SEQ ID NO:5.
88. A DNA comprising a promoter of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene which is substantially represented by SEQ ID NO:7.
89. A DNA comprising a terminator of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene which is substantially represented by SEQ ID NO:8.
90. A gene expression cassette comprising a DNA comprising the promoter as defined in claim 88, a heterologous gene, and the DNA comprising a terminator as defined in claim 89.
91. A recombinant expression vector comprising the gene expression cassette of claim 90.
92. An *Ogataea minuta* strain transformed with the recombinant expression vector of claim 91.

93. An *Ogataea minuta* strain according to claim 92, the strain being from the strain IFO 10746.

94. A process for producing an *Ogataea minuta* strain, which is capable of producing a mammalian type sugar chain represented by the following structural formula (Man₅GlcNAc₂):
Structural Formula 2



comprising a step of disrupting *OCH1* gene (SEQ ID NO:42) in the *Ogataea minuta* strain.

95. A process of claim 94, wherein the *Ogataea minuta* strain is from the strain IFO 10746.

96. A process according to claim 94 or 95, which further comprises a step of disrupting at least one gene selected from the group consisting of a *URA3* gene comprising the nucleotide sequence represented by SEQ ID NO:15, an *ADE1* gene comprising the nucleotide sequence represented by SEQ ID NO:27, an *HIS3* gene comprising the nucleotide sequence represented by SEQ ID NO:99, and a *LEU2* gene comprising the nucleotide sequence represented by SEQ ID NO:107.

97. A process according to any one of claims 94 to 96, which further comprises a step of disrupting at least one gene selected from the group consisting of a *PEP4* gene comprising the nucleotide sequence represented by SEQ ID NO:51, a *PRB1* gene comprising the nucleotide sequence represented by SEQ ID NO:57, and a *YPS1* gene comprising the nucleotide sequence represented by SEQ ID NO:115.

98. A process according to any one of claims 94 to 97, which further comprises a step of disrupting a *KTR1* gene comprising the nucleotide sequence represented by SEQ ID NO:63 and/or an *MNN9* gene comprising the sequence represented by SEQ ID NO:69.

99. A process according to any one of claims 94 to 98, which further comprises a step of introducing and expressing an α -1,2-mannosidase gene from *Aspergillus saitoi*.

100. A process according to claim 99, wherein the α -1,2-mannosidase gene is transferred into the vector of claim 83 and expressed.

101. A process according to any one of claims 94 to 100, which further comprises a step of introducing and expressing a *PDI* gene.

102. A process according to claim 101, wherein the *PDI* gene is a gene (M62815) from *Saccharomyces cerevisiae*.

103. A process according to claim 101 or 102, wherein the *PDI* gene is transferred into the vector of claim 83 and expressed.

104. A process according to any one of claims 94 to 103, which further comprises a step of introducing and expressing a heterologous gene.

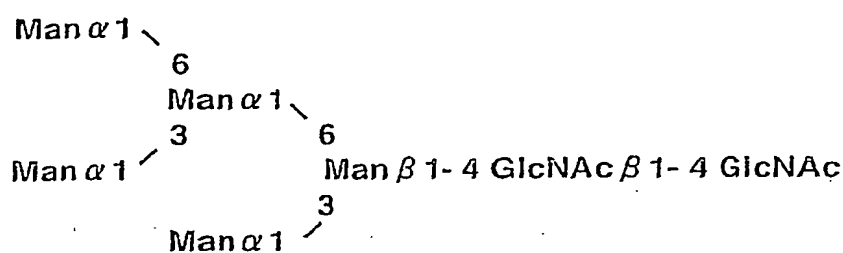
105. A process according to claim 104, wherein the heterologous gene is transferred into the vector of claim 83 and expressed.

106. A process for producing a protein encoded by a heterologous gene, which comprises culturing *Ogataea minuta* produced by the process of claim 104 or 105 in a medium, to obtain the protein comprising a mammalian type sugar chain encoded by the heterologous gene from the culture.

107. A protein comprising a mammalian type sugar chain encoded by a heterologous gene, wherein the protein has been produced by the process of claim 106.

108. A process for producing an *Ogataea minuta* strain, which is capable of producing a mammalian type sugar chain represented by the following structural formula (Man₅GlcNAc₂):

Structural Formula 2



wherein the process comprises the steps of:

disrupting an *OCH1* gene comprising the nucleotide sequence represented by SEQ ID NO:42 in an *Ogataea minuta* strain; and

disrupting a *URA3* gene comprising the nucleotide sequence represented by SEQ ID NO:15 in the same strain; and

disrupting a *PEP4* gene comprising the nucleotide sequence represented by SEQ ID NO:51 in the same strain; and

disrupting a *PRB1* gene comprising the nucleotide sequence represented by SEQ ID NO:57 in the same strain.

109. A process according to claim 108, wherein the *Ogataea minuta* strain is from the strain IFO 10746.

110. A process according to claim 108 or 109, which further comprises a step of disrupting an *ADE1* gene comprising the nucleotide sequence represented by SEQ ID NO:27.

111. A process according to claim 110, which further comprises a step of disrupting a *KTR1* gene comprising the nucleotide sequence represented by SEQ ID NO:63.

112. A process according to claim 111, which further comprises a step of disrupting an *HIS3* gene comprising the nucleotide sequence represented by SEQ ID NO:99.

113. A process according to claim 111, which further comprises a step of disrupting a *LEU2* gene comprising the nucleotide sequence represented by SEQ ID NO:107.

114. A process according to claim 111, which further comprises a step of:

1) disrupting a *YPS1* gene comprising the nucleotide sequence represented by SEQ ID NO:115.

115. A process according to any one of claims 108 to 114, which further comprises a step of introducing and expressing an α -1,2-mannosidase gene.

116. A process according to claim 115, wherein the α -1,2-mannosidase gene is transferred into the vector of claim 83 and expressed.

117. A process according to any one of claims 108 to 116, which further comprises a step of introducing and expressing a *PDI* gene (M62815).

118. A process according to claim 117, wherein the *PDI* gene (M62815) is transferred into the vector of claim 83 and expressed.

119. A process according to any one of claims 108 to 118, which further comprises a step of introducing and expressing a heterologous gene.

120. A process according to claim 119, wherein the heterologous gene is transferred into the vector of claim 83 and expressed.

121. A process for producing a protein encoded by a heterologous gene comprising a mammalian type sugar chain, wherein the process comprises culturing *Ogataea minuta* produced by the process of claim 119 or 120 in a medium to obtain the protein from the culture.

122. A protein encoded by a heterologous gene comprising a mammalian type sugar chain, wherein the protein has been produced by the process of claim 121.